conformations in mutant Htt^{ex1} (Legleiter et al., 2009), Kim et al. (2009) provide direct structural evidence of this, suggesting that, at least in principle, each conformation may seed a unique type of aggregate.

Even if we fully understood how different monomeric conformations of polyQ in Htt^{ex1} lead to various aggregated species, the questions of which species contribute to neurotoxicity and how they do it are still open questions. Kim et al. (2009) propose two general mechanisms for polyQ-mediated toxicity. By one mechanism, the expanded polyQ stretch adopts a de novo conformation that mediates toxicity or is the precursor to a toxic species. By the second mechanism, the expanded polyQ stretch is largely unstructured but presents a very large linear binding surface for proteins with a polyQ affinity. The structures from Kim et al. (2009) leave open the possibility that either mechanism may be correct.

The study by Kim et al. (2009) also provides interesting insight into the relationship between the polyQ stretch and the surrounding sequences in Htt^{ex1}. The N^{17} sequence, which is important for the subcellular localization of Htt^{ex1} and is highly conserved (100% similarity) in all vertebrate species (Atwal et al., 2007), was invariably α-helical in all solved structures. Interestingly, the N^{17} α-helix appears to "bleed" into the C-terminal adjacent polyQ region, causing 1–15 glutamines to participate in the extended α helix (Figure 1A). The structural data from Kim et al. (2009) also hint that the polyQ repeat in Htt^{ex1} may be influenced by the C-terminal polyproline region. Because Htt^{ex1} may be more aggregation prone (and possibly more toxic) when the polyQ region is more compact, it is interesting to speculate whether the polyproline region may serve both its known function as a protein-interaction domain and a less-appreciated function as a protector against polyQ conformational collapse. Indeed, this structural explanation may account for why Htt^{ex1} with the polyproline stretch is less toxic and aggregation prone than Htt^{ex1} without this sequence (Bhattacharyya et al., 2006; Darnell et al., 2007; Duennwald et al., 2006). Thus, N^{17} and polyproline dance partners may keep the Cha-cha-cha-prone polyQ stretch of huntingtin in step, and thereby prevent a toxic conformational stumble.

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Keeping an Eye on Membrane Transport by TR-WAXS

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In this issue of *Structure*, Andersson et al. apply time-resolved wide angle X-ray scattering (TR-WAXS) to follow light-induced conformational changes for both bacteriorhodopsin and proteorhodopsin and probe real-time dynamics at atomic resolution.

Membrane transport proteins perform a multitude of cellular reactions, including energy and signal transduction, regulation of ion concentrations, and transport of metabolites into the cell and noxious substances out. Altered membrane protein function underlies many human diseases, and thus, a deeper understanding of membrane protein structure and dynamics remains a critical objective for basic and medical research. It is well established that membrane transport proteins require distinct temporally regulated structural rearrangements to carry out their biological functions. However, structural details of these dynamic macromolecules have only been studied as snapshots of individual static (and, in most cases, stable) conformations. What is lacking is the ability to capture the transition between these conformations and to probe the role of specific domains and ligands in the process as they proceed through the membrane.

In recent years, our knowledge of membrane protein structure has dramatically increased, providing unforeseen

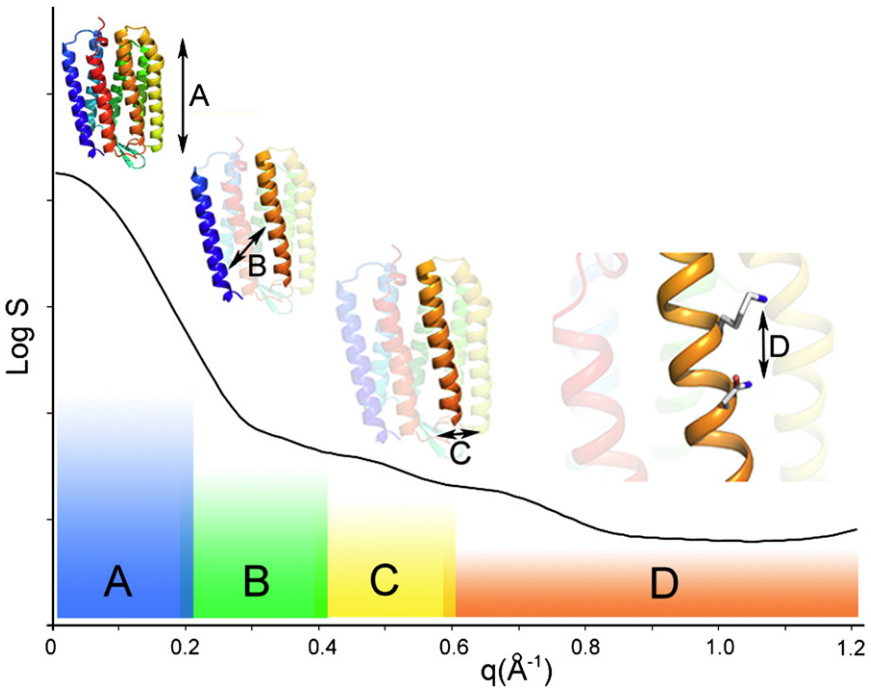

Figure 1. Structural Information Accessible through TR-WAXS
Conformational changes that occur as the protein moves through its reaction cycle are captured by subtracting static protein spectra (unexcited) from protein progressing through its reaction (excited). This difference spectrum can be used to refine a previously resolved 3D structure to fit these conformational changes and further identify distinct time scales in the reaction. The information contained in WAXS data varies with resolution: the overall shape and approximate size of the protein is resolved at $q < 0.2$ Å^{-1}(A, blue shading); a more detailed pattern appears describing the interactions between subunits and or domains ($0.2 < q < 0.4$ Å^{-1}) (B, green shading); interactions between secondary structures ($0.4 < q < 0.6$ Å^{-1}) (C, yellow shading); and interactions between the atoms forming secondary structures ($q > 0.6$ Å^{-1}) (D, orange shading). Here $q = 4\pi\sin(\theta)/\lambda$, where 2θ is the angle between the incident X-ray beam and the detector measuring the scattered intensity, and λ is the wavelength of the X-rays.

abilities to target specific functions of the protein. However, the structures solved are still static. To shed light on movements of the protein, the structural biologist attempts to steer the protein into different conformations through a host of biochemical tricks (e.g., mutations, ligands, inhibitors, truncations, etc). Collection of a composite of structures in such a manner, followed by the incorporation of biochemical, biophysical, and computational methods, allows a portrait of the reaction mechanism to be determined. There is no better poster child for such an exhaustive approach than the light-driven proton pump, bacteriorhodopsin (BR). BR has provided the scientific community with over 20 structures in at least 4 conformations and a seemingly endless amount of biochemical, biophysical, and spectroscopy data, resulting in what is likely the most complete mechanistic understanding of any membrane transport system (Hirai and Subramaniam, 2009; Neutze et al., 2002). It is essential to recognize, however, that this relatively complete picture for BR is the result of three decades of scientific work from hundreds of research laboratories around the world. To be feasible for application to many proteins, a different approach is clearly necessary.

The question then becomes: is there a simpler and more accurate way to capture real-time protein structure dynamics at atomic resolution?

In this issue of *Structure*, Andersson et al. (2009) follow the light driven reaction of BR using time-resolved wide-angle X-ray scattering (TR-WAXS). By expanding the TR-WAXS technique to membrane transport proteins, the investigators not only contribute a major technical innovation to the field of membrane protein dynamics, but also further refine the well-studied BR reaction cycle. TR-WAXS was initially used for monitoring rapid structural rearrangements of small molecules in solution (Ihee et al., 2005; Neutze et al., 2001; Vincent et al., 2009), and more recently was extended to study conformational dynamics on the oxygen transport protein hemoglobin (Cammarata et al., 2008). Unlike traditional spectroscopic methods, WAXS is sensitive to changes in the position of all the atoms in the protein and not simply alterations around a given spectroscopic marker. A typical spectrum captures the scattered intensity from the protein solution as a function of the scattering vector q, where q acts as an atomic ruler (Figure 1). For q values less than 0.2 Å^{-1} ($q < 0.2$ Å^{-1}), the overall shape and approximate size of the protein is observed. Increasing q values provide more detailed structural arrangements: interactions between subunits and/or domains ($0.2 < q < 0.4$ Å^{-1}), interactions between secondary structural elements ($0.4 < q < 0.6$ Å^{-1}), and ultimately interactions between individual atoms ($q > 0.6$ Å^{-1}). Conformational changes occurring in the protein can be identified in TR-WAXS difference data by subtracting the ground state (unexcited) from the protein in an excited state, as it proceeds through the reaction cycle. Most importantly, in terms of the major implications of TR-WAXS, these changes can be monitored as a function of time.

BR is a light driven proton pump that, upon photon absorption, causes a covalently bound retinal to isomerize from an all-*trans* to a 13-*cis* conformation. This isomerization triggers a cascade of conformational changes that drives proton translocation. The details of this mechanism have been probed by numerous biochemical and biophysical techniques but the timing of the primary proton transfer event and the magnitude of conformational changes associated with the photo-cycle remain controversial. To answer these questions, Andersson et al. (2009) visualized real-time helical motion associated with proton translocation by collecting TR-WAXS difference spectra from nonactivated and light-activated BR solution over a time course of 360 ns to 100 ms. Spectral decomposition revealed that the time-scale and transitions through the light-driven cycle

contain three distinct components (early, intermediate, and late).

The authors initiated the BR reaction with a laser pulse and collected WAXS data from delayed X-ray pulses over a 360 ns to 100 ms range. To map these changes at the atomic level, the theoretical scattering curves were calculated from the known three-dimensional structures of BR and refined to the experimental scattering in the three-component system. The early state rapidly decays into the intermediate component in 22 μs, and is characterized by an outward flex on the cytoplasmic portion of helices E and F, and an inward flex of helix C toward the Schiff base. For the first time, conformational changes prior to the primary proton transfer event were revealed, highlighting the sensitivity of TR-WAXS and its application toward characterizing rapid conformational changes in solution. After deprotonation of the Schiff base, the reaction proceeds to the late state, with a time scale of 1.9 ms resulting in a further extension of helices C, E, and F. The late conformation structure, refined against the TR-WAXS data, revealed significantly larger conformational changes than previously reported. Continuing through the reaction cycle, the structure returns to the ground state in 16 ms. This study has not only provided proof of principle for the application of TR-WAXS to membrane proteins, but has significantly complemented previous structural and biophysical studies by refining the order of events and extent of conformational changes during the BR reaction cycle (Figure 2).

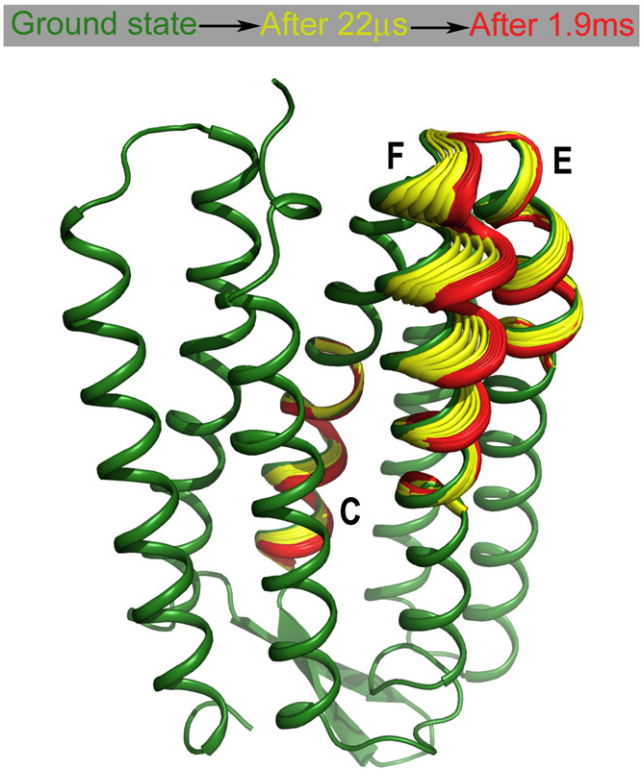

Figure 2. Cartoon Representation of the Light-Induced Conformational Changes occurring in Bacteriorhodopsin
The induced conformational changes are from ground state in green, to the intermediate state after 22 μs in yellow, and to the late state after 1.9 ms in red. The intermediate structures have been generated using rigimol and ipymol.

To demonstrate application to another light-driven proton pump, the authors further extended this study to analyze proteorhodopsin (PR). Again, a three-component system (early, intermediate, and late) provided a complete description of the TR-WAXS data but with a distinctly different time course (τ_1 = 670 ns, τ_2 = 10 us, and τ_3 = 79 ms). Although the motions for PR were less well resolved, the general pattern that emerged exhibited dynamical principles that were shared with BR and thus likely constitute a complete representation of the conserved structural basis for proton pumping. In total, Andersson et al. (2009) were able to capitalize on a technique (TR-WAXS) that monitors fast structural changes with a nanosecond time resolution and overcome a number of experimental challenges to develop the method for use with membrane transport proteins. In doing so, they refined the reaction mechanism of BR by identifying specific conformational changes occurring prior to the primary proton transfer event and further identified appreciably larger conformation changes (throughout the reaction cycle) than have been previously reported. As described by Andersson et al. (2009), TR-WAXS constitutes a powerful new component in the structural biologist arsenal, capable of tracking discrete conformational changes with nanosecond time resolution on well-characterized proteins.

Will TR-WAXS become a technique that is utilized by other researchers to characterize less well-understood systems? For this to happen, there needs to be a concerted effort to improve both hardware and computational methods for application of TR-WAXS to proteins. In particular, algorithms for refining structures to WAXS data are still rather limited and rely solely on rigid body movements. In all likelihood, the actual rearrangements of the protein are far more complex and this highlights the strength of TR-WAXS—it escapes reliance on static structures to capture a reaction mechanism in real time. With further improvements of hardware at synchrotron facilities, this innovation may make possible the use of a single three-dimensional structure in conjunction with TR-WAXS to seamlessly describe the conformational changes throughout the reaction cycle of a protein.

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